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Plant Evolution: Divergent Plants, Divergent Functions for C1HDZ Orthologs**Isabel Monte¹**

Ortholog identification inferred by phylogenetic analyses does not always correlate with functional conservation. The recent functional characterization of the C1HDZ transcription factor in the early-diverging land plant *Marchantia polymorpha* reveals its role in biotic stress responses, contrary to its orthologs in flowering plants.

Plant terrestrialization occurred millions of years ago and required multiple innovations, such as tolerance to desiccation and development of immunity against organisms not present in the aquatic environment. The characteristics of these first land plants has intrigued plant biologists for a very long time. The study of fossils has provided essential insight into plants that became extinct long ago. More recently, genome sequencing of streptophytes and establishment of early-diverging plant models have shed light on the molecular mechanisms underlying terrestrialization. Notably, some of these model plants are bryophytes, which is the sister lineage to vascular plants. Thus, bryophytes have a unique evolutionary position, which we can use to understand plant evolution. Even though bryophytes are fascinating on their own, the power of these models resides in the comparisons we can establish between them and other plant lineages [1]. This will ultimately contribute to inferring the ancestral status of the most recent common ancestor of land plants. Three model species, namely *Physcomitrella patens*, *Marchantia polymorpha* and the recently sequenced *Anthoceros agrestis* represent the three different clades of bryophytes [2–4]. Bryophytes show an alternation of generations where the dominant phase is the haploid gametophyte, in contrast to vascular plants where the diploid sporophytic phase is dominant. Bryophytes undergo asexual reproduction, and can be propagated vegetatively. Among bryophyte models, the liverwort *Marchantia* is becoming increasingly popular due to its amenability to transformation and gene editing, a life cycle completed in three weeks through asexual reproduction, and more importantly, its low gene redundancy. This can be exemplified by the 394 transcription factors (TFs) encoded in the *Marchantia* genome [3] versus more than 1500 TFs present in the most widely used angiosperm model *Arabidopsis thaliana*.

In this issue of *Current Biology*, Facundo Romani, Elizabeta Banic *et al.* [5] report the characterization of MpC1HDZ, the single-copy CLASS I HOMEODOMAIN LEUCINE-ZIPPER (HDZ) TF in *Marchantia*. The HDZ family is present in all streptophytes, whereas in chlorophytes, HDZ sequences exhibit a divergent leucine-zipper domain [6]. In early charophytes and land plants, HDZ family is subdivided into four different classes. In *Arabidopsis*, class 1 HDZ subfamily encompasses 17 members and is involved in leaf and root development, senescence and response to drought [7–9]. To date, there is no comprehensive study aimed at knocking out the whole Class 1 HDZ subfamily and defining the putative specific and overlapping roles of all 17 members in *Arabidopsis*. This represents a long-standing issue regarding specificity and redundancy of multi-copy gene families in many plant species, for which genetic characterization has not been possible. Genome editing by CRISPR/Cas9 has undoubtedly improved this, but an alternative strategy is studying plant species with a lower gene redundancy. *Marchantia*, having one single ortholog with all the features of the Class I HDZ, is thus a promising model for the characterization of this subfamily in embryophytes. The apparent simplicity of *Marchantia* unveils an unexpected discovery: rather than regulating abiotic stress responses, MpC1HDZ was involved in oil body formation, terpenoid biosynthesis and response to herbivory.

Oil bodies, a synapomorphy of liverworts, are specialized organelles that accumulate secondary metabolites as terpenoids or bisbibenzyls [10]. Oil body composition affects the organoleptic properties of liverworts. Several centuries ago, when the doctrine of signatures was a common practice, the bitter taste of liverworts was camouflaged with honey when used to treat different pathologies [11]. Based on our current knowledge, it is very likely that the bitter flavor of the liverwort was due to the compounds stored in the oil bodies. *Marchantia* oil bodies are present in scattered cells referred to as idioblasts. The role of oil body cells has been proposed to be associated with different stress responses including the response to herbivory [10]. Beyond their composition, the formation and function of oil bodies remain largely uncharacterized until now [12].

In this article, Romani and colleagues [5] show that MpC1HDZ is a positive regulator of oil body cell formation. The *Mpc1hdz* knock-out mutants displayed reduced number of oil body cells, and this

phenotype could be complemented by the expression of the wild-type gene. Nutrient starvation and cultivation on non-axenic substrate have been shown to increase oil body cells in *Marchantia* [10]. Nevertheless, *Mpc1hdz* oil body cells remained low even under these inducing conditions. The role of MpC1HDZ in oil body formation was further supported by the use of translational reporter lines, which indicated that MpC1HDZ expression precedes oil body formation. Unlike *Arabidopsis* Class 1 HDZ, MpC1HDZ expression was not altered by different abiotic stresses. *Mpc1hdz* mutant plants performed as wild-type plants under a number of abiotic stresses, namely salt, osmotic, heat and cold stress, UV, nutrient starvation, desiccation and ABA treatment. Notably, the absence of oil body cells did not affect the nutrient starvation response, even though a depletion in nutrients induces the formation of new oil body cells [10]. These results suggest that MpC1HDZ regulates oil body formation, but neither MpC1HDZ nor oil bodies are involved in the response to abiotic stress, contrary to the *Arabidopsis* Class 1 HDZ orthologs.

Oil bodies act as storage organelles for secondary metabolites, which are considered an inducible defense mechanism against different organisms [10]. Therefore, Romani and colleagues explored the role of MpC1HDZ in biotic stress. They challenged *Mpc1hdz* mutant and wild-type plants with the starved pill bugs *Armadillidium vulgare* and discovered that MpC1HDZ was a positive regulator of defense against herbivores. Moreover, they found that MpC1HDZ also regulates the antimicrobial activity of plant extracts. *Mpc1hdz* extracts, but not wild-type extracts, failed to kill the bacterium *Bacillus subtilis*. Transcriptomic analysis revealed that MpC1HDZ regulates the expression of terpenoid biosynthetic genes. Subsequent metabolomic profiling confirmed that terpenoids accumulation is altered in *Mpc1hdz*. This work highlights the role of the single *Marchantia* ortholog of Class 1 HDZ TF in oil body formation, biosynthesis of secondary metabolites and defense against herbivores, and provides the first link between these three processes in *Marchantia*.

Nonetheless, the most important aspect of the MpC1HDZ characterization is the finding that ortholog genes in divergent plant lineages are involved in completely different processes [5]. Alone, the sequence conservation might have predicted a conserved role in abiotic stress responses, but the functional characterization of MpC1HDZ ruled out this hypothesis [5,6]. Phylogenetic analyses and identification of orthologous genes constitute a large fraction of evolutionary studies. Functional characterization involving genetic manipulation is a desirable next step to confirm or discard hypotheses based on sequence conservation. Thus, *Marchantia* is an ideal genetic model to test hypotheses related to functional conservation in land plants [1]. *Marchantia* has therefore been instrumental in showing the functional conservation of several TFs in embryophytes. These studies have shown that land plants' R2R3-MYB TFs are involved in biosynthesis of secondary metabolites; MYCs control jasmonate responses, terpenoid biosynthesis and response to herbivory; ARFs govern auxin responses; RSL and LRL mediate rhizoid/root hair development and FGMYB regulates female gametophyte development [13–20].

In certain cases, the study of these genes in *Marchantia* has uncovered additional functions, such as the role of MpRSL in gemmae development, or the exquisite regulation of MpFGMYB by its antisense gene MpSUF to ensure sexual differentiation [16,20]. Other studies have described a partial functional overlap between *Marchantia* and *Arabidopsis* TFs. For instance, fertility is not controlled by MpMYC, suggesting that MYCs were co-opted for fertility regulation later on in evolution [19]. Class 1 HDZ subfamily represents a different scenario in which these TFs regulate opposite pathways in early-diverging land plants and angiosperms. *Arabidopsis* Class 1 HDZs regulate different developmental processes and response to abiotic stress. Conversely, MpC1HDZ is involved in oil body formation, secondary metabolism and response to herbivores, indicating that the function of Class 1 HDZ is not conserved in land plants. In the future, it will be indeed interesting to pinpoint when Class 1 HDZ acquired new functions during land plant evolution. This question could be tackled by the characterization of Class 1 HDZs in different plant lineages. The work by Romani *et al.* reveals additional roles for Class 1 HDZs, underlies the importance of functional characterization following ortholog identification, and illustrates the need of additional models belonging to different plant lineages to uncover the evolution of this TF subfamily.

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In Brief

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